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I. SALMONELLA/MICROSOME (S/M) MUTATION ASSAY: TA98 ACTIVITY WITH METABOLIC ACTIVATION (+S9) OF IMPACTION TRAPPED (IT) CSC COLLECTED FROM A SERIES OF LOW ALKALOID FILLER CIGARETTES[1]

Strain TA98+S9 was used in the S/M assay to determine the specific activity (S.A.) of IT CSC obtained from a series of blended filler cigarettes. The cigarettes contained either Virginia sun cured or dark air cured tobacco blended with either normal or low alkaloid bright and burley. All of the blended filler CSC samples from the series of CSCs were lower in S.A. than the 100% dark air cured or the 100% Virginia sun cured samples. Substitution of low alkaloid material did not decrease activity. (In one case the low alkaloid significantly increased the activity).

II. S/M TA98+S9 ACTIVITY OF IT CSC COLLECTED FROM 100% BRIGHT CONTROL VERSUS 100% BRIGHT SUPERCRITICAL CO_2 EXTRACTED FILLER[1]

Strain TA98+S9 was used to determine the activity of IT CSC collected from 100% bright control versus 100% bright supercritical CO_2 extracted filler. No significant difference in CSC S.A. could be demonstrated between the 100% bright control and 100% bright supercritical CO_2 extracted filler samples, i.e., supercritical CO_2 extraction of 100% bright filler had no effect on the resulting CSC S.A. as compared to the untreated 100% bright control filler CSC.

III. S/M TA102+S9 ACTIVITY OF IT CSC COLLECTED FROM SIX MODEL CIGARETTES[1]

Strain TA102+S9 was used to determine the activity of IT CSC collected from the following six model cigarettes: 2R1, bright, LTF-5E, burley, RCB, and RL Ref. Four replicate samples/model were tested in order to obtain statistically significant S.A. values.

Results showed that all of the samples were active. RL Ref. and bright CSCs were lower in activity; 2R1 CSC was intermediate in activity; and burley, LTF-5E, and RCB CSCs were higher in activity. No correlation was seen between strain TA98+S9 and strain TA102+S9 results for these model CSCs. Strain TA102 appeared to have a different sensitivity to the model CSCs than did strain TA98. When strain TA102+S9 CSC activity data were qualitatively compared to GDA, L5178Y TK +/-, and IMC activity data for the model cigarettes, no correlation in the data was seen between TA102+S9 and any of these other assays. The

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reason for this observation could be due to the different assay endpoints and /or the different cell types (bacterial versus mammalian used in these assays.

IV. EFFECTS OF STEMS AND/OR RECONSTITUTION ON S/M TA102+S9 ACTIVITY[1]

Strain TA102+S9 was used to determine the effects of stems and/or reconstitution on S/M activity. The effects on strains TA98+S9 and TA100+S9 activity were previously reported.[3] IT CSCs from the following model cigarettes were tested: (1) 75% DBC burley + 25% bright ES (X6D3DAM); (2) RL sheet from #1 (X6D3DFZ); (3) low solubles BW sheet from #1 (X6D3DGA); (4) 50% DBC burley + 50% bright ES (X6D3DBF); (5) RL sheet from #4 (X6D3DNY); (6) 100% bright ES (x6D3CDL); (7) RL sheet from #6 (X6D4CUC); and (8) low solubles BW sheet from #6 (X6D4ATT).

As reported for strains TA98+S9 and TA100+S9, strain TA102+S9 results showed that increased levels of bright ES in the burley-bright ES blend significantly lowered the S.A. of the CSC. In addition, no significant difference in strain TA102+S9 S.A. could be demonstrated between the CSC from any of the parent fillers and the reconstituted sheet made from those fillers.

V. GLUTATHIONE DEPLETION ASSAY (GDA): EFFECTS OF 2R1 CSC ON THE GLUTATHIONE (GSH) LEVELS OF V79 CELLS[4]

An experiment was carried out which showed that more GSH was depleted with fresh 2R1 CSC than 1, 2, or 3 hour old (stored at room temperature) 2R1 CSC.

VI. VALIDATION OF A METHOD TO DETERMINE TRUE TOXICITIES IN THE IMC ASSAY[5]

Experiments were conducted in order to validate a method for the determination of agent-induced toxicity in the IMC assay. 6-Thioguanine (6TG) was utilized to inactivate sensitive (S) cells so that the toxicity of an agent to resistant cells can be evaluated. Several 6TG pre-incubation times were tested to determine the minimum 6TG pre-incubation time necessary to prevent colony formation of 5E5 S cells. Results showed that a pre-incubation time of 44 hrs reduced S colonies to a tolerable level (12 colonies/5E5 cells), while further pre-incubation (68 hrs) reduced S colonies to the quite acceptable level of 2 colonies/5E5 cells. A test is in progress to confirm these results and to determine whether 1 or 2 rinses are needed to reduce the concentration of residual 6TG in dishes to a non-toxic level.

VII. REFERENCES

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